Comparison Between Vaginal Tampon and Cervicovaginal Lavage Specimen Collection for Detection of Human Papillomavirus DNA by the Polymerase Chain Reaction

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The aim of the study was to compare the accuracy of self-administered vaginal tampon (VT) specimens for the detection of human papillomaviruses (HPVs) with that of cervicovaginal lavage specimens (CVL). Two hundred seventy-four paired VT and CVL specimens were collected prospectively from women at risk of sexually transmitted diseases. Specimens were treated and amplified with the polymerase chain reaction (PCR). Each woman served as her own control. One hundred and forty-four of 272 (52.9%) CVLs and 159 of 271 (58.7%) VTs contained HPV DNA sequences (correlation of 88%). The sensitivity and specificity of vaginal tampons reached 93.9% (138/147) and 80.5% (99/123), respectively. HPV typing results were concordant for 99 negative paired samples and 114 paired samples positive for the same type(s) (correlation of 78.9%). It is concluded that these sampling methods collect cells from different areas of the genital epithelium, highlighting the importance of further assessment of the comparative predictive value of HPV detection in each sample. ***J Med Virol 51: 42–47, 1997.*** © 1997 Wiley-Liss, Inc.

KEY WORDS: PCR; HPV; vaginal tampons; sample; STD

INTRODUCTION

Human papillomaviruses (HPVs) are associated with genital tract malignancies [Franco, 1991]. Large scale prospective studies are needed to define the natural history of HPV infection and disease progression in the general population. The most sensitive method for HPV testing and typing of clinical specimens is the polymerase chain reaction (PCR) [Bauer et al., 1991; Schiffman et al., 1993]. Currently available sampling methods for obtaining epithelial cells from the genital tract for HPV testing have drawbacks. Biopsies allow the evaluation of the presence of HPV in discrete lesions but are not representative of the entire cervical epithelium. Although cervical scrapes or brushings collect epithelial cells from a wider area of the ectocervix, they provide an insufficient quantity of DNA for repeat testings, contain only superficial epithelial cells, and are significantly less sensitive than cervicovaginal lavage [Burk et al., 1986; Morrison et al., 1992; Vermund et al., 1989; Goldberg et al., 1989]. Cervicovaginal lavage specimen lends itself well to PCR techniques for HPV analysis [Coutlée et al., 1992].

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Most of these specimen collection techniques require a gynecological examination. Self-administered cervicovaginal lavage is easy to perform, but patient compliance appears to limitate the value of this sampling method [Morrison et al., 1992]. In contrast, vaginal tampon specimens are self-administered non-invasive sampling methods [Fairley et al., 1992] that could facilitate greatly the screening of large numbers of participants for natural history studies or could be useful to identify HPV-infected women without a gynecological examination. In one study in which DNA extraction was carried out on all samples, results for HPV detection obtained with vaginal tampons correlated highly with those of cervical scrapes [Fairley et al., 1992]. However, the integrity of DNA was not evaluated with a human gene amplification control, and only few HPV types were screened.

Our study was aimed at comparing results of HPV and β-globin DNA sequence detection in cervicovaginal lavages with those obtained with self-inserted vaginal tampon specimens. Each woman thus served as her own control. The incomplete concordance between these sampling methods found in this study requires further prospective evaluation of the predictive value of detecting HPV in various types of specimens.

METHODS

Patient Protocol

The study population included women participating in the Canadian Women's HIV Study. This multicentre study, which has ethics committee approval of each participating institution, is being conducted across Canada to evaluate the relationship between HPV infection, HIV infection, and cervical disease using cross-sectional, cohort, and descriptive methodologies. For the purpose of this analysis, most samples came from women undergoing baseline visits, although some samples were obtained from repeat visits conducted 6 months after enrollment. Women seropositive for HIV-1 (200 women) and seronegative for HIV-1 (30 women) were eligible. Seronegative women were recruited from STD clinics if they had had more than three lifetime sexual partners. After the goal and implications of the study were explained and questions answered to their satisfaction, participating women signed an informed consent form.

Sample Processing

Before the physical examination, the participant was asked to insert and immediately withdraw a vaginal tampon (Meds regular, Johnson & Johnson). The vaginal tampon was placed into a sterile jar containing 50 ml of 10 mM Tris-HCl, pH 7.5, 50 mM EDTA, and 150 mM NaCl [Fairley et al., 1992]. During the pelvic examination, a cervical scrape was obtained for a Papanicolaou smear. A cervicovaginal lavage was then done with 10 ml of sterile PBS sprayed on the ectocervix with a syringe [Burk et al., 1986; Vermund et al., 1989] and aspirated from the posterior vaginal fornix. Specimens were refrigerated within 1 hour. The delay between sampling and processing never exceeded 7 days.

Cells were pelleted after centrifugation at 2,500 rpm for 10 min at 4°C. The cell pellet was resuspended in 500 μl of 10 mM Tris, pH 8.3. Cell suspensions were lysed with Tween 20 and NP-40, and were digested with proteinase K as previously described [Coutlee et al., 1992]. Cell lysates were stored at −70°C until amplified.

DNA Amplification

Gene amplification was done without knowledge of cytological diagnosis, HIV infection status, or results of previous HPV testing. Cell lysates were amplified in duplicate with L1 consensus HPV primers MY09/MY11 under standard conditions [Bauer et al., 1991; Hildesheim et al., 1994]. Negative, weakly positive (10 HPV-18 DNA copies), and strongly positive controls (HPV types 6/11,16,31,33,35,39,45), were included in each run. PCR products were spotted onto a nylon membrane [Sambrook et al., 1989]. Amplification of a 268 pb β-globin DNA fragment was accomplished on each sample with PC04/GH20 [Bauer et al., 1991] in parallel reactions in order to control for DNA integrity and for the absence of amplification inhibitors. Products were separated by electrophoresis on a 2% ethidium-stained agarose gel, and visualized on a UV transilluminator. Samples generating a visible 268 bp band were tested for HPV DNA, while DNA from the other samples was extracted with phenol-chloroform and precipitated with ethanol. One μg of extracted nucleic acids was reamplified for β-globin and HPV. If positive for at least one of these reactions, the sample was considered adequate for PCR analysis. If negative for both β-globin and HPV, then the specimen was considered inadequate for HPV testing. Measures to avoid false-positive reactions due to contamination were followed strictly [Kwok and Higuchi, 1989].

Detection of HPV Amplified Products

PCR products spotted onto nylon membranes were first hybridized with an HPV generic probe mixture under low stringency conditions. The generic probe mixture was generated by amplification in separate reactions of HPV-16, HPV-18, HPV-31 plasmids with type-specific nested primers [Guerrero et al., 1992] and ^{32}P-deoxynucleotides. Amplified nested L1 fragments were mixed and used as a generic probe that efficiently detects common genital types [Guerrero et al., 1992; Collins, 1993]. All membranes were also reacted under stringent conditions with type-specific oligonucleotide probes end-labeled with ^{32}P-ATP [Bauer et al., 1991], against HPV types 6/11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, and 58 [Hildesheim et al., 1994; Bauer et al., 1992; Schiffman et al., 1993]. Samples positive with the generic probe but negative with all the type-specific probes, contained untyped HPV.

Statistical Analysis

The sensitivity and specificity of vaginal tampon specimens were calculated considering the cervicovaginal

lavage as the gold standard [Goldberg et al., 1989; Burk et al., 1986; Vermund et al., 1989]. The crude percentage of agreement between sampling methods was the percentage of pairwise samples where HPV DNA or typing results were identical. The unweighed kappa statistic [Fleiss, 1981] was calculated to adjust for chance agreement between sampling methods. In general, a kappa value above 0.75 represents excellent agreement beyond chance, while values below 0.40 represent poor agreement beyond chance [Fleiss, 1981]. In between values (0.40–0.75) correspond to a fair to good agreement beyond chance. Paired *t*-tests were used to test for comparisons between sampling methods of the number of different HPV types detected per sample. Proportions were calculated from contingency tables and compared using chi-square tests with Yate's correction for continuity.

RESULTS

Initially, 230 women provided 574 samples. Of the 166 women with available PAP smear results, 82 (49.4%) had normal smears, 45 (27.1%) had benign atypia, 32 (19.3%) had squamous dysplasia, and 7 (4.2%) had changes consistent with condyloma (Hankins C. et al., Preliminary results from the Canadian Women HIV Study, presented at The HIV infection in Women Conference, Washington, February, 1995). Clinical data not included in the current analysis will be reported elsewhere. Twenty-six samples were rejected from analysis: 12 vaginal tampons were collected without cervicovaginal lavage, two cervicovaginal lavages were collected without vaginal tampon, two samples were spilled during transportation, and 10 samples were biopsies. Overall, 224 women (30 HIV-seronegative and 194 HIV-seropositive women) provided 274 paired specimens (first visit, 224 and follow-up visit, 50). Five specimens (0.9%) from four women were inadequate for PCR analysis (three vaginal tampons and two cervicovaginal lavages). Thus, one woman could not be evaluated for HPV infection and five pairs of specimens could not be compared for HPV results. Of the 543 samples adequate for PCR analysis, 73 (13.4%) required DNA extraction, including 40 (14.7%) of 272 cervicovaginal lavage and 33 (12.2%) of 271 vaginal tampon specimens ($P = 0.47$).

Combining results from both sampling methods, 140 (62.8%) of 223 women were infected with HPV at baseline or follow-up including, 12 (40%) of 30 HIV-seronegative women and 128 (66.3%) of 193 HIV-seropositive women. Of the 543 specimens that could be analyzed with PCR for HPV detection and typing, 303 (55.8%) contained HPV sequences. Untyped HPVs were detected in 76 samples (14.0%): 37 (13.6%) of 272 cervicovaginal lavage and 39 (14.4%) of 271 vaginal tampon specimens ($P = 0.89$). The distribution of HPV types was similar for each sampling method ($P > 0.2$ for all comparisons, data not shown). HPV-16, -52, -53, and -58 were the most frequently encountered types (Fig. 1). Eighty-one isolates (26.7%) were high-risk types (types 16 or 18), 129 (42.6%) were intermediate-risk types (31,33,35,39, 45,51,52,53,56,58), and 93 (30.7%) were unknown or low-risk types (6/11, untyped) [Schiffman et al., 1993].

Multiple HPV types were detected in 108 (19.9%) of the 543 specimens from 53 (23.8%) of 223 women. These included 51 cervicovaginal lavage and 57 vaginal tampon samples. HPV-positive specimens contained in average 1.7 ± 1.2 (± one standard deviation) HPV types overall: 1.7 ± 1.2 for cervicovaginal lavage and 1.8 ± 1.3 types for vaginal tampon specimens ($P = 0.33$). Samples with more than one HPV type contained in average 3.1 ± 1.3 types: 3.0 ± 1.2 for cervicovaginal lavage and 3.1 ± 1.3 types for vaginal tampon specimens ($P = 0.68$). Individual samples contained at most seven different HPV types. In the 59 pairs of samples with a least one specimen containing more than one HPV type, results were similar between both methods of sampling for 40 women (67.8%), completely different for one (1.7%), discordant with cervicovaginal lavage containing a greater number of types than vaginal tampon specimens for 5 (8.5%), discordant with vaginal tampon containing more types than cervicovaginal lavage specimens for 13 women (22.0%).

Overall, 144 (52.9%) of 272 cervicovaginal lavage and 159 (58.7%) of 271 vaginal tampon specimens adequate for PCR analysis contained HPV DNA by PCR ($P = 0.21$). There was a good correlation for the presence of HPV DNA comparing pairs of samples obtained at the same visit (Table I). Because four women provided at least one inadequate specimen, only 270 pairs of samples could be evaluated. HPV DNA was detected in 138 pairs while 99 pairs tested negative for HPV (87.8% crude correlation: k = 0.76). Correlations were identical in HIV-infected and uninfected women (data not shown). The sensitivity and specificity for detection of HPV DNA sequences using vaginal tampon specimens were therefore 93.9% (138/147 pairs) and 80.5% (99/123 pairs), respectively. In the paired samples, HPV DNA was detected more often in the vaginal tampon alone than in the cervicovaginal lavage alone (24 pairs [8.8%] vs. nine pairs [3.3%], $P = 0.01$).

When HPV typing results were considered, PCR results were identical for 213 pairs (9 negative paired samples and 114 paired samples positive for the same type[s] identified, for a correlation of 78.9% and a kappa value of 0.60). Correlations were similar in HIV-infected and uninfected women (data not shown). For five pairs, both samples of the same pair contained different HPV types. For 16 pairs of samples (5.9%), cervicovaginal lavage only was positive (nine pairs) or contained at least one additional type as compared to the vaginal tampon (seven pairs). For 36 pairs (13.3%), the vaginal tampon specimen only was positive (24 pairs) or contained at least one additional type as compared to the cervicovaginal lavage (12 pairs). This ability of vaginal tampon specimens to permit more frequent detection of HPV DNA or of a greater number of types per sample than cervicovaginal lavage, was statistically significant (36/270 vs. 16/270, $P = 0.005$). The sensitivity and specificity of vaginal tampons decreased to 84.4% (114/135)

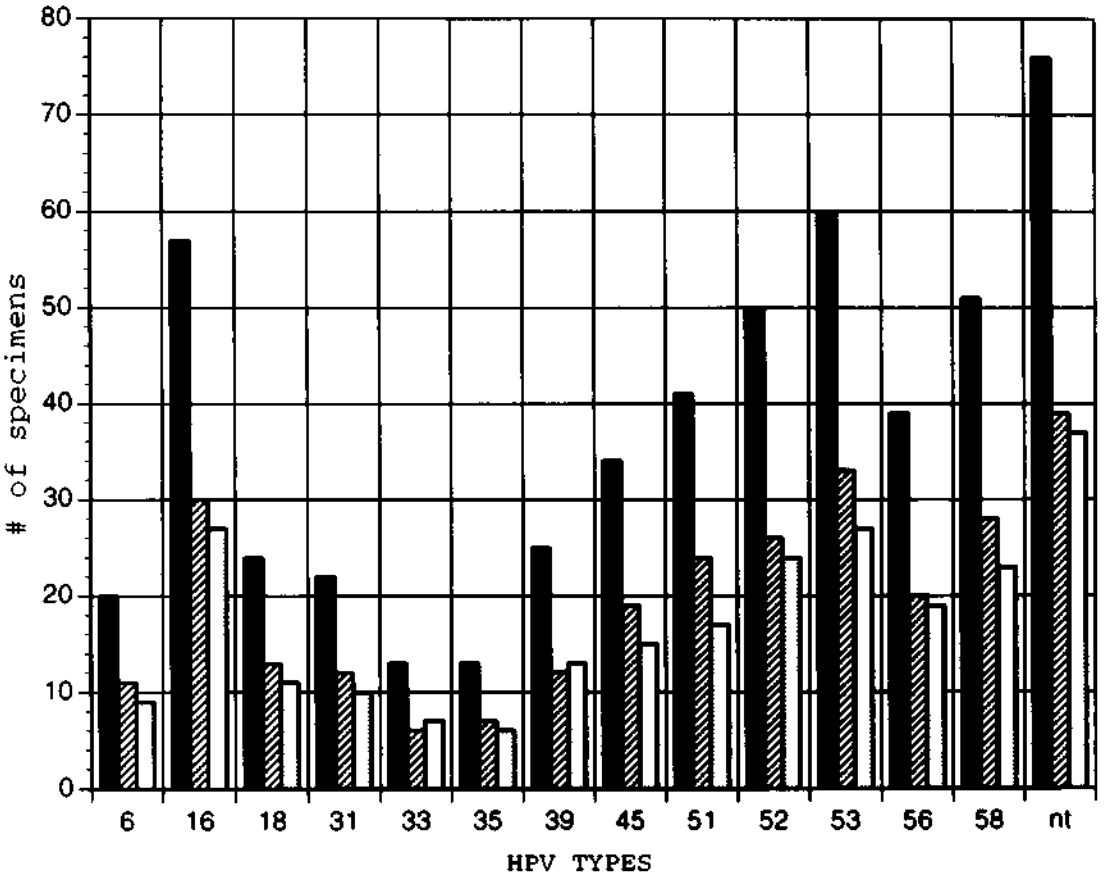

Fig. 1. Distribution of HPV types in cervicovaginal lavages (CVL) □ and vaginal tampons (VT) ▨ and combined samples ■. nt is for untyped HPV. Overall 303 specimens were positive, including 144 CVLs and 159 VTs.

TABLE I. Comparison of HPV DNA Detection in Cervicovaginal Lavages and Vaginal Tampons by the L1 Consensus PCR Assay*

	Cervicovaginal lavage results	
Vaginal tampon results	Positive	Negative
Positive	138 (114)	24 (36)
Negative	9 (16)	99 (99)

*Comparison of HPV DNA detection in cervicovaginal lavages and vaginal tampons by the L1 consensus PCR assay. Two hundred seventy paired samples were treated as described in the Methods section and amplified with a consensus PCR assay for HPV detection. Numbers in parenthesis are results obtained when typing was considered. Other numbers in the table are thus obtained considering only the presence of HPV DNA without considering typing of HPV DNA.

and 73.3% (99/135), respectively, when typing results were considered.

Repeat paired samples were obtained for 51 women, of whom 50 provided pairs of samples adequate for PCR analysis. HPV detection and typing was concordant between both cervicovaginal lavages sampled at a 6-month interval for 30 (60%) of 50 samples, with the repeat sample containing more HPV types than the first sample for 13, fewer types for three, and completely different types for four women. HPV typing was concordant between both vaginal tampons sampled at a 6-month interval for 27 (54%) of 50 samples, while the repeat sample contained more types for 13, fewer types for four and completely different types for six women. The proportion of concordant results was similar for both sampling methods ($P = 0.11$).

DISCUSSION

In this study, vaginal tampons were compared with the cervicovaginal lavage, the best means for detecting cervical HPV infection, [Burk et al., 1986; Vermund et al., 1989; Goldberg et al., 1989]. Overall, only 1% of samples were unsuitable for PCR analysis. Lysis with protein digestion was found to be adequate treatment for cell suspensions with only 12.2% of tampon specimens requiring DNA extraction for efficient amplification of β-globin gene sequences. In our study, there was good correlation (87.8%) for the presence of HPV DNA between vaginal tampon and cervicovaginal lavage from paired specimens.

This finding is consistent with previous studies which

demonstrated a correlation of 88% between cervical scrapes and vaginal tampon specimens [Fairley et al., 1992], and of nearly 90% between vulvar or self-administered vaginal swabs and cervical scrapes [Bauer et al., 1991; Moscicki, 1993]. When results from HPV typing were compared, sampling methods were concordant for nearly 80% of pairs, a net improvement from the first description of vaginal tampon use as means of specimen collection for detecting HPV. In this latter publication, the correlation between scrapes and tampons decreased to 52% when typing was considered [Fairley et al., 1992]. This lower correlation could be explained by the inclusion of a smaller number of specimens tested and by the use of scrapes that are recognized to be less sensitive than cervicovaginal lavage. We also controlled for the quality of the specimens by performing β-globin assays. In contrast to previous reports on the use of non-invasive sampling techniques for HPV detection, we studied a large number of samples collected consecutively. We also detected a wide range of HPV types and analyzed each type individually and not broad categories of types [Moscicki, 1993; Fairley et al., 1992; Goldberg et al., 1989].

In our study, HPV DNA was detected significantly more often in vaginal tampons only than in cervicovaginal lavages only. Overall, genital HPV infections in our population of women could have been confirmed with PCR using vaginal tampon specimens in 93.9% of cases as compared with 84.2% with cervicovaginal lavage samples. Differences in the detection rates and typing of HPV DNA between cervicovaginal lavages and vaginal tampons could be explained in part by differential viral shedding, localized HPV infection, incomplete sample collection, or sampling of cells from different sites [Fairley et al., 1992; Moscicki, 1993; Morrison et al., 1992]. Sampling of the ectocervix, endocervix, and vaginal fornices could more readily detect multicentric disease that would be missed by biopsy or cervical scrapes.

However, these results could also reflect the effect of tampon sampling prior to the cervicovaginal lavage for all women. This limitation in our study design could not be avoided. It was impractical to attribute randomly the sequence in which the sampling methods would be done, since the presence of PBS remaining in the genital tract after the lavage could influence the value of vaginal tampon. All samples were tested in duplicates. Results from duplicate testings for each sampling method were concordant for all samples, illustrating the reproducibility of the PCR assay used.

As described previously [Reeves et al., 1989; Schneider et al., 1992; Vernon et al., 1994; Rosenfeld et al., 1992; Moscicki et al., 1993], HPV detection was highly variable over a 6 month period using cervicovaginal lavage and tampon samples. The importance of HPV infection in a population composed mainly of HIV-infected women is demonstrated by the high detection rate of HPV DNA. Nearly two thirds of women were found to have HPV infection during the study period. An important proportion of HPV detected with consensus PCR assays could not be typed with known HPV probes [Bauer et al., 1991]. Our results also underscore the importance of HPV types that have been considered in recent studies [Hildesheim et al., 1994], such as types 51, 52, 53, 56, and 58. Most of the HPV isolates were high or intermediate-risk types. Multiple HPV types were detected in 47.6% of the specimens, a finding that has been reported in studies using consensus primers and a large panel of type-specific probes [Hildesheim, et al. 1994].

It is concluded that vaginal tampon specimens offer an easy method for obtaining adequate cellular material from the genital tract for HPV analysis with PCR. Correlation between vaginal tampon and cervicovaginal lavage samples was high for the presence of HPV DNA and still reached nearly 80% when typing results were considered. Relationship of HPV findings to disease or disease progression requires further assessment, since each sampling technique collects cells from different areas of the genital epithelium. Definitive determination of the use of vaginal tampons necessitates a prospective evaluation of the predictive value of HPV results for disease progression or persistence of HPV infection. Such a longitudinal study is currently underway in our cohort of women.

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